

ORIGINAL RESEARCH ARTICLE

**Comparative Studies on Growth Performance of Probiotic Supplemented Rohu (*Labeo rohita*) Fingerlings**

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**ABSTRACT**

This study reports the effect of the gram positive probiotic strains on growth of rohu (*Labeo rohita*) fingerlings. The growth was assessed by morphometric measurements, feed conversion ratio, feed conversion efficiency and protein efficiency. The formulated fish feed was added with probiotics and introduced in fish aquaria @ 3% body weight in three forms; formulated feed without probiotic (T<sub>1</sub> control), feed with commercial probiotic (T<sub>2</sub>) and feed with gram positive probiotics (T<sub>3</sub>). In 90 days experiment, morphometric measurements were made fortnightly. T<sub>3</sub> shows significant growth performance than those of T<sub>2</sub> and control groups. The FCR, FCE and PER ratio also shows significant change in T<sub>3</sub> when compared with other groups. These results clearly indicate the importance of probiotic feed technology in aquaculture science. The potential of gram positive probiotics were identified and gram positive probiotic feed was formulated for commercial fish farming, Carp culture Rohu (*Labeo rohita*).

**Key words:** Probiotics, Carp.

**INTRODUCTION**

In Asia, an average, almost 30 per cent of total protein intake is derived from fish. Fish is a highly nutritious food, containing high amount of protein with high biochemical value for humans. Fish is a principal source of animal protein for over half of the global population. Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut. They are called 'friendly bacteria' or "good bacteria." They are used as complementary and alternative medicine (CAM). Probiotic therapy helps to treat several gastro intestinal illness. Probiotics have already become a significant direction as an alternative to antibiotic treatment for aquaculture and have been commercially available as feed or water additives in pond water (Moriarty 1997; Boyd and Gross, 1998; Verschuere *et al.*, 2000; Wang *et al.*, 2005). Recently FAO has designated the use of probiotics as a major agent for the improvement of aquatic environmental quality (Subasinghe *et al.*, 2003). The term probiotic, originating from the Greek words "Pro" and "bios" means "for life", and was firstly created by Lilley and Stillwell in 1965 as "the substances secreted

by one microorganism, which stimulated the growth of another." Later the definition of probiotic has been gradually changed and could be combined and referred to as "living microorganism mono or mixed culture in sufficient number with or without by products, leading to benefit of host health by improving intestinal balance and of environment (Sperti, 1971; Parker, 1974; Fuller, 1992 and 1997; Havenaar and Hirs. In't Veld, 1992; Salminen 1996; Schaafsma 1996; Gatesoupe, 1999; Tannock, 1999; Gismondo *et al.*, 1999; Verschuere *et al.*, 2000; FAO, 2001; Irianto and Austin, 2002). Among probiotic bacteria for aquaculture *Bacillus* spp. *Lactobacillus* spp. and *Streptococcal* spp. are more widely used and proved to enhance aqua product health with no visible side effects (Vaseeharan, 2003; Ziaei Nejad *et al.*, 2006; Balca'zar and Rojas-Luna, 2007; Gomez and Shen, 2008; Tseng *et al.*, 2009; Liu *et al.*, 2010). *Bacillus* probiotic supplement in shrimp feed (Rengpipat *et al.*, 1998; Balca'zar, 2003; Tseng, 2009) or culture water (Moriarty, 1998; Gullian *et al.*, 2004; Ziaei-Nejad, 2006) is

expanding rapidly with an increasing number of studies demonstrating immune stimulation, antimicrobial activities and competitive exclusion. In this study the five gram positive probiotic has been extensively studied and characterized to ensure its nutritive value for fish culture Rohu (*Labeo rohita*). *Labeo rohita*, *Cirrtina mirgala* are most preferred farmed fish sp., because of their growth and higher acceptability to the consumers (Javaid, 1990; Javeel *et al.*, 1993). For the present study, *L. rohita* (Rohu) was selected, due to its rapid growth attainment of large size, quality of flesh and consumer preference. It is a freshwater herbivore. Rohu (*L. rohita*) is known as water column feeder.

## MATERIALS AND METHODS

### Description of the Study Area

The fingerlings for this experiment were collected from RK fish farm at Orathanadu in Thanjavur dist. at Tamilnadu, India.

### Experimental Design

*Labeo rohita* fingerlings weighing (1+2 g) were grouped into three each with 100, as T<sub>1</sub>, (basal diet without probiotics), T<sub>2</sub> (commercial probiotics with gram negative strains) and T<sub>3</sub> (Formulated gram positive probiotics). Water physicochemical parameters were maintained by (AOAC and APHA). The fishes were fed twice at 9.00 hrs and 16 hours daily, according to body weight.

### Preparation and Packaging of Feeds

The collected feed ingredients were grounded thoroughly and served to pass through 0.5 mm mesh. The ingredients were mixed according to the formulation. T<sub>1</sub> with Basal diet (Fish meal 18%, Groundnut oil cake 18% Sesame oil cake – 16%, Rice or wheat bran 16%, Tapioca flour 16%, Fish oil 3%, 0.5% Essential aminoacids and vitamins) with approximate composition of 39% protein, 24% carbohydrate, 11% lipid and 0% ash, T<sub>2</sub> with basal diet coated with commercial probiotics and T<sub>3</sub> with basal diet coated with formulated gram positive probiotics. The ingredients were put into the manually operated pellet machine for the preparation of pelleted feed of size 2 mm.

### Feeding and Sampling

The experimental feeds were supplied daily morning at 9.00 AM and evening at 5.00 PM at a rate of 3 per cent of the body weight. Sampling was done at an interval of 15 days to adjust the feeding rate and the weight of the fish was measured with portable electronic balance (Model AK-3000H AFD).

### Morphometric Measurements of the Fingerlings

At every 15 days are the fishes were measured for wet body weight. After obtaining the data, five fish per treatment were sampled, wet weight gain was calculated.

**Wet weight gain (g) = Final weight (g) – Initial weight (g)**

Feed conversion ratio (FCR) was calculated

**FCR = Total feed consumption (g) / weight gain (g)**

The percent feed conversion efficiency (FCE %) for each group was calculated.

**FCE% = wet weight gain (g)/feed consumed (g) × 100**

### Proximate Analysis of Feed and Faeces

Feed and fecal matter are collected dried at 105°C (oven till consistent weight) weighed amount of dried feed (0.5 g) and feces (0.25 g) were taken and homogenized. The sample was immersed in 4 ml of 0.89% cold saline solution and homogenized for 1 minute in motor driven homogenizer at 8000 rpm. Homogenate was centrifuged at 4900 rpm for 45 minutes. The clear supernatant was separated and used for determining total protein.

From the difference of total protein (mg/g) of respective feeds and faeces, protein intake was calculated.

Protein efficiency Ratio (PER) was then calculated as:

**PER = (wet weight gain/feed protein intake) × 100.**

### Statistical Analysis

Experimental data are presented as mean ± SE and were analyzed with one way ANOVA followed by Tukey's test to compare the means between individual treatments at a significant level of P<0.05.

## RESULTS

### Effect of Formulated Gram Positive Probiotic Feed on Growth Performance of *Labeo rohita* Fingerlings

The control T<sub>1</sub>, commercial probiotic T<sub>2</sub>, and formulated probiotic fishes were fed with the sterile formulated feed (% b.wt). The control feed with basal diet (T<sub>1</sub>) and the T<sub>2</sub> with commercial probiotic and T<sub>3</sub> with formulated probiotic for 90 days. The feed input and recovery of faecal matter and unconsumed feeds are shown in (Table 1 & 2). The fishes from each of the triplicate an aquarium for each experiment was sampled at 15 days intervals and accordingly the total feed per aquarium administered was decreased (Table 1). The progressive decrease in total amount of feed given paralleled decreasing trends for all the three groups. When faeces and

unconsumed feeds were recorded (Table 2). The probiotic feed given to the both experimental groups. (3% body wt), turned out to be significantly different at the last two sampling periods as compared to the respective control values.

This indicated more growth of fishes fed with probiotic feed (Fig 1). Interestingly at the last sampling period, recovered faeces and unconsumed feed showed significantly decreased values for both the experimental groups as compared to the control values (Table 1).

**Feed Conversion Ratio/Efficiency**

When feed conversion ratio (FCR) and percent feed conversion efficiency (FCE%) were worked out the fishes of T<sub>2</sub> showed significantly high body weight gain at third phase, while total feed

input for both the experimental groups showed significant increase over the control values at accomplishment of the experiment (phase VI) FCE% of T<sub>3</sub> than, the other group at last phase of the experiment are indicative of growth promoting effects of probiotic supplemented feed (Fig 2).

**Protein Efficiency Ratio**

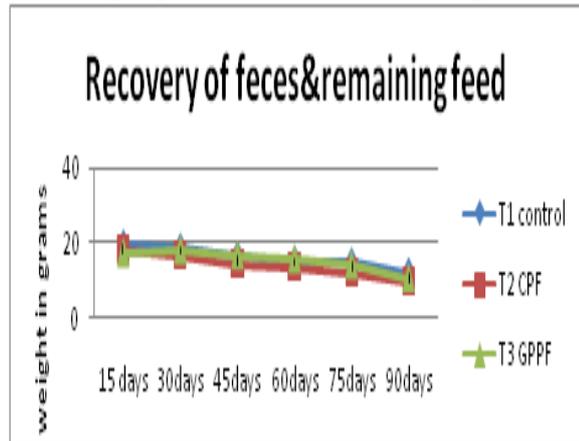
Protein efficiency ratio (PER) were found significantly less for T<sub>2</sub> and T<sub>3</sub> as compared to control (T<sub>1</sub>) at phase I of the experiment. However at third and fourth phases, the experimental groups had significantly higher PER as compared to PER as compared to the respective controls. The probiotic supplement at last phase showed significantly higher PER as compared to control value (Fig 3).

**Table 1: Input of fish feed (g) (3% b.wt) administered to control and experimental groups of *L. rohita* at different phases**

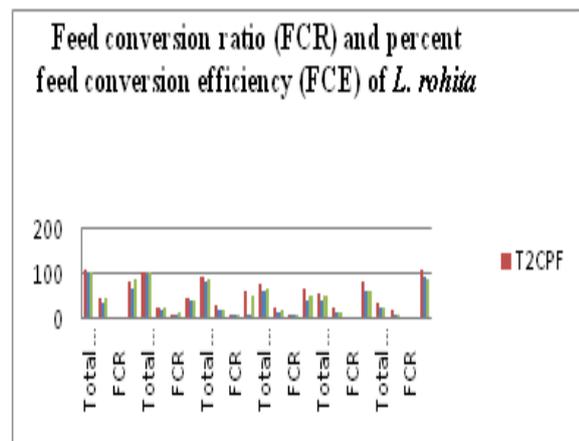
Phase (day)	No. of fish	Control	Commercial probiotics	Formulated probiotic
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
I (15 days)	100	71.35 ± 0.87	71.00 ± 1.69	71.55 ± 2.35
II (30 days)	90	70.35 ± 0.57	71.00 ± 0.69	71.00 ± 2.00
III (45 days)	75	67.20 ± 1.43	68.35 ± 1.67	68.40 ± 1.65
IV (60 days)	60	56.30 ± 2.15	59.35 ± 1.18	55.98 ± 3.59
V (75 days)	45	45.20 ± 1.40	50.20 ± 1.41	48.90 ± 0.84
VI (90 days)	30	32.15 ± 0.73	38.00 ± 1.15	36.40 ± 0.61

All values represent means of 3 triplicate ± S.E.M. Values are significantly different at P ≤ 0.5 at single factor analysis of variance.

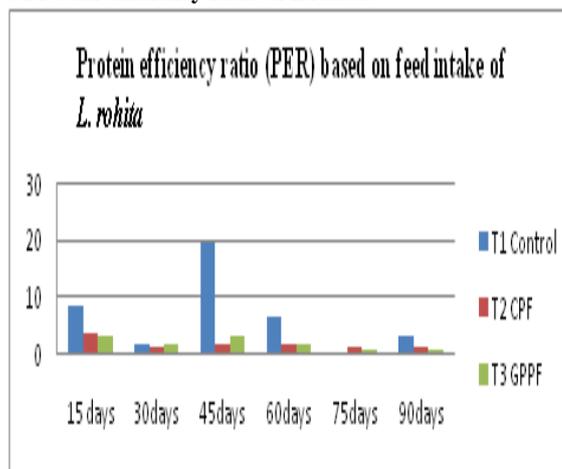
**Fig 1: Recovery of faeces and remaining feed in control and experimental groups of *L. rohita* within different phases**



**Fig 2: Feed conversion ratio/efficiency of *L. rohita***



**Fig 3: Protein efficiency ratio of *L. rohita***



**DISCUSSION**

Aquaculture development has been considered a very rich source of high biologic value protein diets to ever growing human population. Consequently the sector has developed strategies in various countries to improve fish health and fish growth. Among these strategies, the more promising is the use of probiotics. In this investigation the commercial probiotics treated fish (T<sub>2</sub>) as well as the formulated probiotics treated fishes (T<sub>3</sub>) were analyzed for their potential growth promoting effects on rohu (*L. rohita*) fingerlings. Regarding the growth effects of commercial and formulated probiotic feed,

significantly higher levels of growth assessing parameters found for the fishes fed the experimental feeds as compared to the control groups clearly demonstrate the potential of the reported probiotics. Conclusively 18.52 and 12.06 g higher body weight gain, 3.36 and 4.56 FCR, 119.06 and 89.50 FCE%, were recorded for T<sub>2</sub> and T<sub>3</sub> respectively at the last phase of experiment. These results suffice to advocate the beneficial role of probiotics. This claims for the present study has emerged from the foundations laid down by majority of the growth assessing parameter levels. Many authors have commented on the usefulness of administration of probiotics (Gatesoupe, 1999; Gomez Gil *et al.*, 2000; Robertson *et al.*, 2000; Nikoskelainen *et al.*, 2001; Siuta Cruce and Goulet, 2001).

Several worker have described benefits of probiotics to the host that include the improvement in nutrition by the detoxification of potentially harmful compounds in feeds, the hydrolysis of potentially indigestible components in the diet by hydrolysis enzymes including amylases and proteases resulting into increased protein and sugar and decreased fiber levels, the production of vitamins, such a biotin and Vitamin B<sub>12</sub> (Sugita *et al.*, 1991; 1992; Fuller, 1992; Smoragiewicz *et al.*, 1993; Balagopalan, 1996; Sugita *et al.*, 1996; Hoshina *et al.*, 1997). It appears pertinent here to refer that formulated gram positive probiotic is simpler and consequently (potentially) less expensive (Solis-Pereira *et al.*, 1993; Maldonado *et al.*, 1998; Diaz-Godinez, 2001).

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